The effect of vegetation structure and abiotic variables on oviposition-site selection by amphibians

by

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Abstract

Assessing restoration success for pond-breeding amphibians frequently focuses on hydrology, water quality and vegetation, while neglecting the requirements of amphibians that use the restored areas for breeding. Both biotic and abiotic conditions can influence oviposition-site selection of amphibians that do not provide parental care. This study examines how vegetation structure and abiotic variables affect oviposition-site selection by amphibians. The goal of my study was to better understand the requirements of pond-breeding amphibians. In 2017, I surveyed egg masses in four ponds at the Sunshine Coast Botanical Garden in Sechelt, B.C. I identified 667 egg masses of four native amphibian species that varied in abundance and species richness among ponds. I recorded five biotic variables (i.e., vegetation cover, vegetation type, stem density, stem diameter, and canopy closure) and two abiotic variables (i.e., water depth and solar radiation) at egg-mass sites and random sites where no egg masses were detected. Logistic regression analysis with backward elimination revealed that stem count ($p = 0.008$) and water depth ($p = 0.0001$) significantly influenced oviposition-site selection. The results also showed that higher stem density and shallower water depth increased the likelihood of egg masses being present. My study indicated that quantifying stems in the water column characterized vegetation density better than estimating percent cover of vegetation. Shallow areas that have structurally complex vegetation might provide an advantage for the offspring by increasing refuge, food resources, and favourable thermal conditions for egg development. Hence, restoration projects could incorporate vegetation structure and shallow areas in their pond designs to potentially increase the abundance and diversity of amphibian communities, thereby contributing to successful restoration projects.

Keywords: amphibians, oviposition, *Rana aurora*, *Pseudacris regilla*, *Ambystoma gracile*, *Ambystoma macrodactylum*, vegetation structure, abiotic variables, ecological restoration

Dedication

I am dedicating this thesis to my father, Hans-Hermann Paetow (1931.09.08 – 2016.11.30), whose unwavering support and spirit stays with me and continues to encourage me to pursue my goals and fulfill my dreams.

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List of Acronyms

BCIT	British Columbia Institute of Technology
CI	Confidence Interval
df	Degrees of Freedom
EC	Electric Conductivity
EM	Egg Mass
FN	False Negatives
FP	False Positives
GPS	Global Positioning System
M	Mol
NO-EM	No Egg Mass
PAR	Photosynthetically Active Radiation
PPFD	Photosynthetic Photon Flux Density
SD	Standard Deviation
SE	Standard Error
SFU	Simon Fraser University
TN	True Negatives
TP	True Positives

Glossary

Abiotic	Not derived from living organisms
Ambystomatidae	Family of mole salamanders (genus *Ambystoma*)
Anura	Order of amphibians comprising frogs and toads
Biotic	Derived from living organisms
Canopy closure	Proportion of the sky hemisphere obscured by vegetation when viewed from a single point
Canopy cover	Area of the ground covered by a vertical projection of the canopy
Caudata	Order of amphibians comprising salamanders and newts
Confusion matrix	A table used to describe the performance of a classification model on a set of test data for which the true values are known
Hatchling	Juvenile amphibians that have recently emerged from their egg
Hylidae	Family of the tree frogs and their allies (e.g., chorus frogs)
Larva	Immature form of amphibians (also referred to as tadpole in frogs)
Natural selection	Variation in traits of organisms that provide a reproductive advantage that is passed to the next generation leading to increased fitness of the offspring
Oviposition	Egg-mass deposition
Photosynthetically active radiation	Solar radiation in the spectrum from 400 - 700 nm that photosynthesizing organisms process for photosynthesis
Ranidae	Family of the true frogs
Refuge	Concealment cover (e.g., to hide from predators) and shelter cover (e.g., to regulate temperature)
Tadpole	see Larva
Trait	Distinguishing characteristic or feature of an organism
Urodela	An order of amphibians comprising salamanders and newts (also called Caudata)
Vegetation cover	The proportion of the ground (or pond-surface area) concealed by a vertical projection of the vegetation
Vegetation structure	The overall morphology and architecture, or structural complexity, of the vegetation (e.g., vertical layer of plants)
Vegetation type	Life form that gives a plant its character

1.0 Introduction

1.1 Background

Concern for the population status of many amphibians has increased efforts to restore breeding sites for amphibians (Calhoun et al. 2014, Soomets et al. 2016). Restoration projects, however, often lack clear goals or adequate techniques to gauge restoration success (Brown et al. 2012, Denton and Richter 2013, Calhoun et al. 2014). Assessing restoration success frequently focuses on hydrology, water quality and vegetation, while neglecting the requirements of amphibians that use the restored areas for breeding (Purrenhage and Boone 2009, Brown et al. 2012).

Based on the principles of natural selection, one would expect that amphibians would lay their eggs in places that maximize the fitness of the individual. Natural selection means that favourable variation in traits of organisms provides a reproductive advantage that is passed to the next generation leading to increased fitness of the offspring (Darwin 1861). Oviposition-site selection might be of particular advantage for amphibians that do not provide parental care and whose offspring have reduced mobility early in their life stage (Groezinger et al. 2012, Buxton and Sperry 2017). Recent studies have hypothesized that both biotic and abiotic conditions in the aquatic environment of ponds and in the terrestrial environment surrounding ponds influence oviposition-site selection by amphibians (Indermaur et al. 2010, Brown et al. 2012, Groezinger et al. 2012). The vegetation structure, however, that amphibians require in ponds to optimize oviposition sites, has not been well documented.

Various studies have examined the terrestrial and aquatic vegetation surrounding pond margins. Most aquatic studies used line-intercept transects (Egan and Paton 2004), plot sampling (Groezinger et al. 2012, Denton and Richter 2013, Rowe and Garcia 2014, Chandler et al. 2015) or visual estimates (Burne and Griffin 2005, Pearl et al. 2005) below the high-water mark of ponds to assess percent vegetation cover and vegetation type (e.g., submergent, floating, emergent, and woody shrub species). Researchers also determined pond canopy cover or canopy closure (Egan and Paton 2004, Groezinger et al. 2012, Denton and Richter 2013, Chandler et al. 2015), forest-mediated light (Halverson et al. 2003) and water depth to bottom substrate (Licht 1969, Egan and Paton 2004, Rudolf and Roedel 2005, Groezinger et al. 2012).

Available information suggests that percent vegetation cover might have a positive effect on egg-mass deposition (Egan and Paton 2004) and community composition of amphibians (Burne and Griffin 2005, Shulse et al. 2012). This positive influence might be attributed to vegetation characteristics (Egan and Paton 2004) or vegetative complexity (Burne and Griffin 2005, Purrenhage and Boone 2009). Vegetation structure generally varies among and in ponds and can influence amphibian behaviour and population dynamics, leading to changes in species diversity (Purrenhage and Boone 2009). Denser vegetation structure might increase potential sites for egg attachment, while providing refuge and food resources to emerging larvae (Purrenhage and Boone 2009). Vegetation types used for egg attachment have been described by various researchers (Licht 1969, Schaub and Larsen 1978, Richter and Azous 1995, Egan and Paton 2004, Pearl et al. 2005, Calhoun et al. 2014), and might influence egg-deposition behaviour. While the size of the attachment substrate might influence oviposition-site selection by amphibians, stem size has not commonly been reported. Canopy closure (Egan and Paton 2004, Lesbarrères et al. 2010, Denton and Richter 2013) and availability of photosynthetic light (Halverson et al. 2003) also have been linked to changes in community composition. Changes in canopy closure, light and water depth might be associated with changes in temperature (Freidenburg and Skelly 2004) and can affect oviposition-site selection by amphibians in different ways. Many studies have measured water depth in ponds to determine hydroperiod (Pearl et al. 2005, Lesbarrères et al. 2010, Denton and Richter 2013, Calhoun et al. 2014, Hossack 2017) or littoral (i.e., shallow) areas in ponds (Porej and Hetherington 2005).

The requirements of amphibians in relation to vegetation structure, light and water depth, however, have largely been assessed at the pond scale. In contrast, few studies have evaluated pond vegetation structure or abiotic variables directly at oviposition sites (Egan and Paton 2004, Rudolf and Roedel 2005, Purrenhage and Boone 2009).

1.2 Research objective

Here, I examine how vegetation structure and abiotic variables might affect the selection of oviposition sites by amphibians. Characterizing these variables in ponds directly at oviposition sites is useful for identifying the factors that influence oviposition-site selection by amphibians. These data could increase our understanding of the conditions pond-breeding amphibians require in the early reproductive stage, and, hence, maximize species' abundance and diversity. Wetland and pond restoration could benefit from these data because these factors could be incorporated into wetland restoration and management potentially leading to increased restoration success.

1.3 Research hypotheses

Based on my review of the literature, I am testing the following specific predictions:

1. Amphibians select oviposition sites in areas of higher vegetation structural complexity (i.e., higher percent vegetation cover and higher stem density) because these areas provide increased food and refuge for the offspring.

2. Amphibians select vegetation types and plant-stem diameters for egg attachment to increase the chance that egg masses remain in place and attached to the stem for the duration of egg development (e.g., to optimize temperature and light).

3. Anurans (i.e., frogs) select oviposition sites in areas with open canopy and higher solar radiation; whereas urodeles (i.e., salamanders) select oviposition sites in areas with increased canopy closure and lower values of solar radiation.

4. Amphibians select shallow areas less than or equal to 0.5 m in depth over deeper areas (> 0.5 m) for oviposition because these areas have warmer temperatures and therefore optimize egg development (e.g., growth rate).

2.0 Methods

2.1 Study site

I conducted my study at the Sunshine Coast Botanical Garden in Sechelt, 70 km northwest of Vancouver, B.C. (**Figure 1**). This 14-ha area served as a food farm between 1947 and 1980, and a tree farm until its purchase by the Sunshine Coast Botanical Garden Society in 2009. The botanical garden is in the eastern very dry Coastal Western Hemlock biogeoclimatic subzone (Demarchi 2011). This subzone is characterized by warm, dry summers with associated long growing seasons and moist, mild winters (Green and Klinka 1994). The site is in a rural area that contains a mixed forest predominated by young western red cedar (*Thuja plicata*), red alder (*Alnus rubra*), salmonberry (*Rubus spectabilis*), willow (*Salix* spp.) and sword fern (*Polystichum munitum*).

Figure 1. Location of the 14-ha study site at the Sunshine Coast Botanical Garden, northwest of the town of Sechelt, B.C. The imagery date is 14 May 2014 (Google Inc. 2018). The study was conducted in 2017.

The site lies on a plateau that has a high groundwater table. My study site comprised four ponds located 75 m to 350 m apart. Historically, three of the ponds (Frog Pond, North Pond and Towhee Pond) were built for irrigation of the farm. Based on aerial imagery (University of British Columbia 2017), Frog Pond (located in the northeast portion of the site) was constructed between 1957 and 1967, North Pond (located in the north) was built between 1976 and 1980, and Towhee Pond (located in the south) was built in 1982. The fourth pond (Dragonfly Pond, located near the center) was created as an ornamental in 2015. The ponds varied in size from 203 m^2 to 2,041 m^2. I estimated pond sizes using the measuring tool in Google Earth Pro (Google Inc. 2016).

The riparian vegetation around each pond varies. Frog Pond is semi-open and surrounded by trees (e.g., *Thuja plicata* and *Alnus rubra*), shrubs, grasses, and bare ground. The pond margin has been maintained through selective cutting of trees and shrubs and mowing of the grass/herbaceous vegetation on top of the bank. The margins of North Pond and Towhee Pond have relatively dense riparian vegetation consisting of *T. plicata*, *A. rubra*, abundant shrubs (e.g., *Rubus spectabilis*, *R. ursinus*, and *R. armeniacus*), and ferns (e.g., *Polystichum munitum*). Maintenance of North Pond has included brush-cutting and mowing on its banks, while Towhee Pond has been left mostly untouched except for clearing of branches along the adjacent trail to the north. Dragonfly Pond is open, and has been planted with aquatic, emergent and riparian vegetation (e.g., *Typha latifolia*, *Carex* spp., *Juncus* spp., *Potamogeton* spp. and *Schoenoplectus acutus*). The low-growing riparian vegetation around Dragonfly Pond consists of ornamental shrubs and herbaceous plants. In-pond vegetation of Frog Pond, North Pond and Towhee Pond include woody species (e.g., *Salix* spp. and *A. rubra*) and, to a lesser extent, emergent plants (e.g., *Carex* spp., *Iris* sp., and grasses) and aquatic plants (i.e., *Potamogeton* spp.).

2.2 Sampling design

2.2.1 Egg masses

Egg-mass surveys are an effective way to monitor amphibian populations (Crouch and Paton 2000, Egan and Paton 2004). Female frogs usually lay one egg mass (clutch) per season (Perrill and Daniel 1983, Crouch and Paton 2000, Egan and Paton 2004, Jones et al. 2005, Groezinger et al. 2012), whereas female salamanders deposit between one and four egg clutches (Petranka 1998, Egan and Paton 2004, Faccio 2011).

I conducted egg-mass counts along systematic transects to identify amphibian species and relative abundance of egg masses in each pond. I visited each pond three times between 18 March and 28 April 2017, corresponding with the breeding phenology of amphibians at the site. I detected egg masses by walking the shoreline and entering littoral areas of the ponds, scanning the water surface with polarized glasses. I used (8x42) binoculars to confirm egg masses that were located further (up to 5 m) from shore. I was not able to survey at depths below 1.2 m. I used the survey yielding the most egg masses for analysis to prevent double-counting.

I recorded the shoreline location from which I detected the egg masses using a hand-held GPS (Garmin GPSMap 62S) and took photographs of the general area of oviposition. I quantified the depth of each egg mass below the water surface (water surface to edge of egg mass [m]). I identified the developmental stages of select egg masses (Gosner 1960, Harrison 1969) to determine the timing of my next site visit. I verified species based on Corkran and Thoms (1996).

2.2.2 Vegetation structure

I conducted vegetation sampling directly at egg-mass sites (EM sites; June 2017), and at randomly selected sites where I did not observe egg masses during any of the surveys (NO-EM sites; June and July 2017). I confirmed EM sites using the GPS waypoints and photographs recorded in March/April, and observations of remnant (hatched) egg-masses that were still present in June.

I identified zones along each pond where I had not observed egg masses during egg-mass surveys (i.e., GPS coordinates absent). In these zones, I walked systematic transects along the shore, turning perpendicular to the shore (towards the pond) at random stops, and then entered the pond for a random distance. Random distances for turns and stops were based on a random-number calculator (numbers between 1 m and 10 m along the shore and between 1 m and 5 m into the pond, in 0.1-m increments). When the distance into the pond was too long (i.e., pond was too deep or distance exceeded past the pond center), a new number was generated.

I measured five variables to describe the structural complexity of the vegetation at EM sites, and NO-EM sites: percent vegetation cover, canopy closure, vegetation type, stem quantity, and stem diameter. I selected my variables according to local vegetation characteristics, previously published field studies (Egan and Paton 2004, Lesbarrères et al. 2010, Groezinger et al. 2012, Denton and Richter 2013, Holzer 2014), and based on the effort required to measure them. Several studies used “canopy cover” and “canopy closure” interchangeably (Werner and Glennemeier 1999, Calhoun et al. 2014, Chandler et al. 2015). Both methods, however, have been clearly defined: canopy cover is “the area of the ground covered by a vertical projection of the canopy”, whereas canopy closure is “the proportion of the sky hemisphere obscured by vegetation when viewed from a single point” (Jennings et al. 1999). I assessed canopy closure based on (Jennings et al. 1999).

I estimated percent vegetation cover in 1-m^2 plots (made of a frame from pre-measured PVC pipe) centered over each egg mass or group of egg masses on the pond surface. I defined vegetation cover based on (Robinson and Bolen 1984, Jennings et al. 1999). On a few occasions where multiple egg masses were still present in the plot, I used one cover estimate for the entire 1-m^2 plot. This avoided overlapping plots and repeat measurements of the same vegetation cover. At NO-EM sites, I centered the 1-m^2 plots on the randomly determined location. I estimated canopy closure at each plot location by taking readings with a convex spherical densiometer in each of the four cardinal directions (Model-C, R. E. Lemmon Forest Densiometers).

I used 25-cm^2 subplots inside each 1-m^2 plot to determine vegetation type, number of stems and stem diameters. I placed the subplot centered over the egg mass (or group of egg masses) or, at NO-EM sites, in the center of the 1-m^2 plot. Measurements in each subplot included the water column from the water surface to the pond substrate. I identified the vegetation type in each subplot. I classified the vegetation types, based on physical structure or species, into twigs (i.e., *A. rubra* and *Salix* spp.), herbaceous aquatic (i.e., *Potamogeton* spp.), herbaceous emergent (i.e., *T. latifolia*, *Carex* spp., *Juncus* spp., *Schoenoplectus acutus*, and grasses), and herbaceous riparian (i.e., *Rubus spectabilis* and *R. ursinus*). In the case where multiple vegetation types were present in the same subplot, I used the predominant type for analysis or, in absence of a predominant type, recorded all species and classified the vegetation type as variable. In the case where a remnant egg mass was present, I also identified the attachment substrate.

I quantified the stem density by counting the number of stems in the water column at each subplot. In addition, I measured the diameter of each stem that intersected the subplot by taking the measurement at the center of the stem's length inside the subplot. In subplots that had more than 25 plant stems, I measured a random number of stem diameters (using a random number calculator) in one randomly selected quarter of the subplot.

2.2.3 Abiotic measurements

At each EM site and each NO-EM site, I quantified two abiotic variables, water depth and solar radiation. I measured water depth from surface to bottom substrate (in metres). I quantified photosynthetically active radiation (PAR) by measuring the photosynthetic photon flux density (PPFD, in $\mu M/m^2s$) at each plot. PAR is the solar radiation in the spectrum between 400 and 700 nm that photosynthesizing organisms (e.g., plants) process for photosynthesis (Machado and Reich 1999). This range corresponds closely with the range of light visible to the human eye. I took measurements in each cardinal direction using a quantum meter (Apogee MQ-200).

I collected additional data on water levels, water chemistry, and water temperature. In each pond, I installed one water-level gauge in April/May 2017 when water was at a maximum level (i.e., full pool). Gauges consisted of wooden stakes with about 2 m of metal measuring-tape screwed onto the sides. I used a hammer to install the gauges into the pond substrate at similar locations (south or south-west side) in each pond. I recorded a full-pool reading in May and re-recorded water levels once per month to calculate monthly changes until October 2017. I took one final reading in mid-December 2017, after substantial precipitation had occurred. I considered the water-level changes when I measured abiotic variables (e.g., water depth) in June and July and corrected for the difference with water levels measured during egg-mass surveys.

I measured pH, water temperature (°C), and electric conductivity (µS/cm) in-situ on multiple occasions between mid-March and mid-July (Oakten pH/EC Tester). I also quantified dissolved oxygen (mg/L) once at the end of April (YSI Professional Plus handheld multi-parameter meter).

2.3 Statistical analysis

Logistic regression is a statistical modelling tool that predicts the likelihood ratio of a binomial outcome in the presence of more than one explanatory variable (Sperandei 2014). I used a logistic regression with backward elimination in R (R Core Team 2015) to examine the likelihood of seven explanatory variables representing vegetation structure and abiotic conditions (**Table 1**) to elicit a binomial response (presence or not detected) in one dependent variable (egg mass).

Table 1. Description of the dependent and independent variables assessed in this study in 2017 (Sunshine Coast Botanical Garden, Sechelt, B.C.), their data type and data range, and the code used (in R) for each variable. Variables were analyzed at the plot level.

Variable	Data Type / Range	Description
EM.Presence	Binary 1/0	Egg mass present (1) or not detected (0) at each plot
Veg.Cover	Integer 0 - 60	Percent vegetation cover (%) estimated for each plot
Veg.Type	Character	Woody or herbaceous vegetation types present for each plot. Character classes include: woody (i.e., willow or alder twigs), herbaceous riparian (i.e., salmonberry and trailing blackberry), herbaceous emergent (i.e., Tule, sedge, rush, cattail, grass, and various), and herbaceous aquatic (i.e., pondweed).
Num.Stems	Count 0 - 400	Number of stems counted (or estimated) in the water column at each plot, representing stem density
Stem.Dia	Numeric 0.1 - 160.00 (mean 0.3 – 68.8)	Diameter of stems (mm) that intersect the water column at each plot (and mean stem diameter for each plot)
Depth.Bot	Numeric 0.09 - 1.78	Depth of water (m) to substrate at the pond bottom for each plot
Canopy.Close	Numeric 0.16 - 100.00 (mean 0.42 - 99.74)	Percent canopy closure (%) calculated from densiometer reading for each plot in the four cardinal directions (and mean canopy closure for each plot)
PPFD	Numeric 0 – 2106 (mean 0.25 – 2102.5)	Photosynthetic photon flux density ($\mu M/m^2 s$) measured with quantum meter for each plot in the four cardinal directions (and mean PPFD for each plot)

In logistic regression, the likelihood of the response can be expressed as a function, or model, of the explanatory variables:

$$\mathrm{EM.Presence} = \beta 0 + \beta 1 * (X1) + \beta 2 * (X2) + \beta 3 * (X3) + \ldots + \beta n * (Xn) + \varepsilon$$ **Equation 1**

This equation estimates the likelihood value (i.e., 1 ‘present’ or 0 ‘not detected’) for egg-mass presence given the constants and the seven explanatory variables (Sperandei 2014). In the equation, “β_0 to β_n”, represent the regression coefficients (i.e., constants) associated with the reference group (i.e., β_0) and the explanatory variables (i.e., X_n), and “ε” represents the error term.

I determined which independent variables contribute significantly to the model using the backward elimination technique for parameter selection. Backward elimination refers to the process where the regression starts with all variables in the model (i.e., the ‘full’ regression model) to determine the likelihood of a ‘1’ response then works backwards by eliminating each variable that is non-significant (i.e., the change in the fit of the model with or without an individual variable is not statistically significant, $p>0.05$). I used the glm() function in R (R Core Team 2015) to run the logistic regression.

I tested how good the model is as a predictor of my data, using the “caTools” package in R (Tuszynski 2014). I split the data set, at random, into training set (80%) and testing set (20%) and applied the testing set to predict the model accuracy. I calculated the model accuracy (in percent) from the resulting confusion matrix.

3.0 Results

3.1 Egg masses

Across the four study ponds (referred to as A, B, C, and D), I detected 667 egg masses of four native amphibian species (**Table 2**). The four species belong to two Orders (*Anura* and *Caudata*) and three families (*Ranidae*, *Hylidae*, and *Ambystomatidae*). Species richness and overall abundance of egg masses were highest in ponds B and C (4 species) and lowest in pond A (2 species).

Table 2. Egg-mass abundance by pond and by species, including totals. Ponds are: Dragonfly (A), Frog (B), North (C), and Towhee (D). The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

	Pond Identification (Area)				
Species	A (203 m^2)	B (967 m^2)	C (665 m^2)	D (2041 m^2)	**Egg Masses**
R. aurora (red-legged frog)	54	19	35	69	177
P. regilla (northern Pacific treefrog)	44	2	11	1	58
A. gracile (northwestern salamander)	0	281	60	6	347
A. macrodactylum (long-toed salamander)	0	55	30	0	85
Total	**98**	**357**	**136**	**76**	**667**

Egg-mass density (calculated as abundance per m^2) of the four species varied among the ponds (**Figure 2**). While the smallest pond (i.e., Dragonfly, A) had the egg masses of the fewest number of species (n = 2), this pond had the highest density of anuran egg masses (*R. aurora* and *P. regilla*). Frog Pond (B) had the highest density of *A. gracile* and *A. macrodactylum* egg masses, in addition to being one of the ponds containing all four species. Although Towhee Pond (D) is the largest of the ponds, its egg-mass density was the lowest.

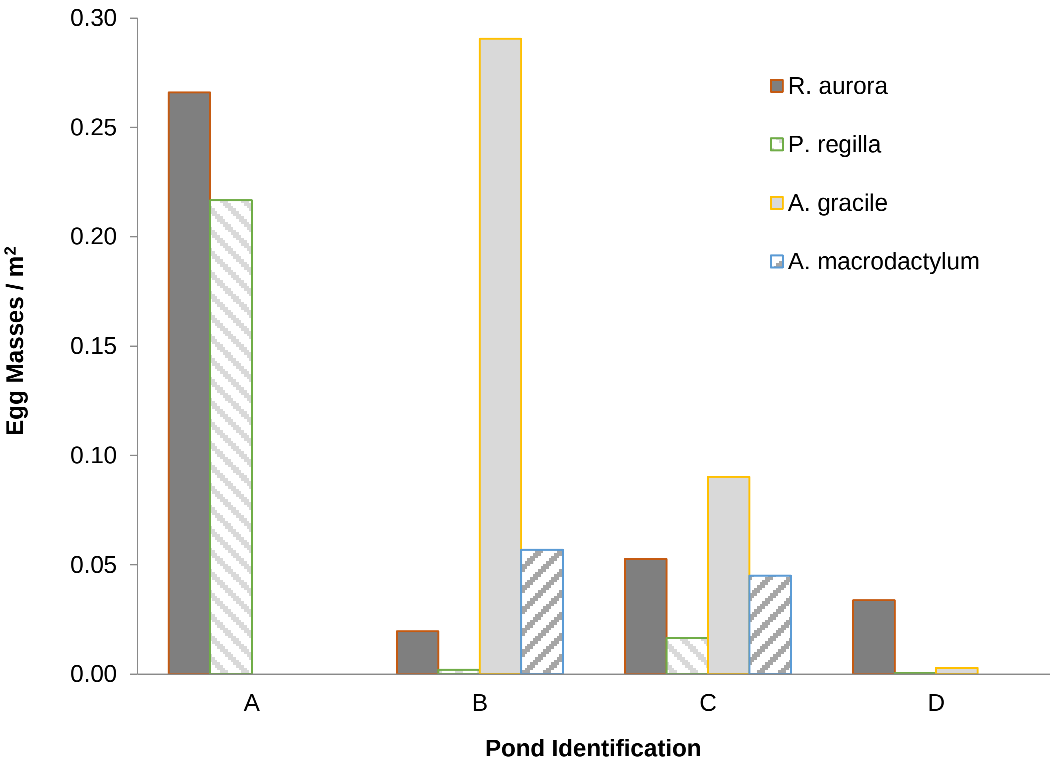

Figure 2. Egg-mass density, by species, for each study pond. The species are *R. aurora*, *P. regilla*, *A. gracile*, and *A. macrodactylum*. The ponds are Dragonfly (A), Frog (B), North (C), and Towhee (D). The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

3.2 Egg-mass sites

Based on my observations at 82 plots, the water depth at egg-mass sites (mean ± 95% CI) was 0.44 ± 0.03 m (range 0.09 – 0.94 m, n = 96). The mean water depth was similar for sites with *A. gracile* (0.41 ± 0.04 m, range 0.20 – 0.81 m, n = 38) and *A. macrodactylum* egg masses (0.41 ± 0.07 m, range 0.30 – 0.60 m, n = 9) (**Figure 3**). *Pseudacris regilla* deposited egg masses at sites with shallower mean depth (0.31 ± 0.04 m, range 0.2 – 0.45 m, n = 10), while *R. aurora* deposited egg masses at sites with greater mean depth (0.52 ± 0.05 m, range 0.09 – 0.94 m, n = 39), when compared to the other species. Egg masses were submerged at a mean depth (± 95% CI) of 7 ± 1 cm below the water surface (range 0 – 25 cm, n = 90) with the depth varying between species. Mean egg-mass depth below surface was 3 ± 2 cm for *R. aurora* (range 0 – 25 cm, n = 39), 7 ± 3 cm for *P. regilla* (range 3 – 13 cm, n = 6), 9 ± 2 cm for *A. gracile* (range 0 – 20 cm, n = 36), and 13 ± 5 cm for *A. macrodactylum* (range 5 – 20 cm, n = 9).

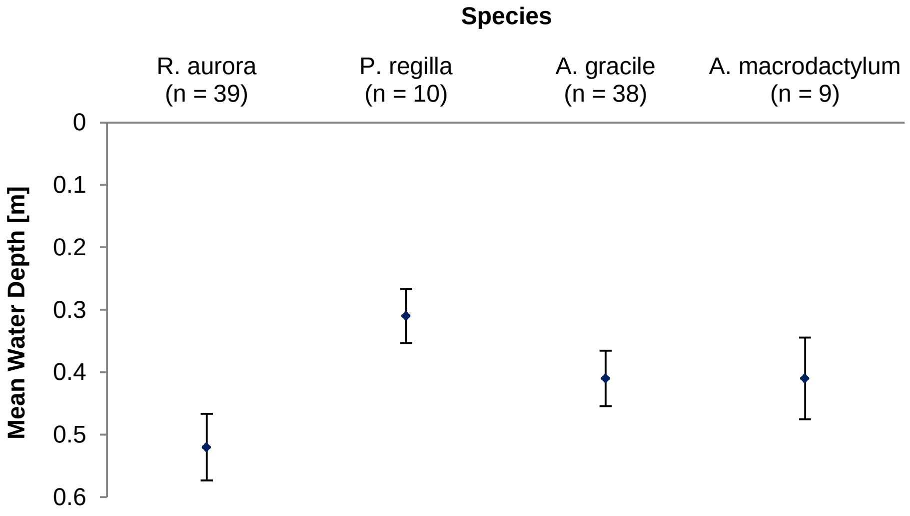

Figure 3. Mean (± 95% CI) water depth, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

Egg masses were attached to live or dead, woody or herbaceous vegetation (**Table 3**). I recorded egg masses of *R. aurora* (n = 28) predominantly among (*Salix* or *Alnus*) twigs (29%) and *S. acutus* stems (25%), and to a lesser extent *Typha*, *Carex*, *R. spectabilis*, *R. ursinus* and grasses (ranging between 7% – 11%). The attachment substrate of *P. regilla* egg masses (n = 8) also varied; *R. ursinus* stems (25%) and twigs (25%) were predominant and *Carex* and *Juncus* (both 13%) secondary. *A. gracile* egg masses (n = 37) were mainly attached to twigs (84%), while lesser attachment substrates included *R. ursinus*, *R. spectabilis* and grasses (e.g., *Phalaris arundinacea*). *A. macrodactylum* egg masses (n = 9) occurred on twigs (89%), except for one occurrence on *R. ursinus*.

Table 3. Broad vegetation classes and species recorded at egg mass sites for four amphibian species. Vegetation classes and species are differentiated into predominant (**+**), secondary (o) and minor (-).The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

Vegetation Class / Species	**Amphibian Species**			
	R. aurora	*P. regilla*	*A. gracile*	*A. macrodactylum*
Herbaceous Aquatic	-	-	-	-
Herbaceous Emergent	+	o	o	-
Herbaceous Riparian	o	+	o	o
Woody Twigs	+	+	+	+
Salix spp.	+	+	+	+
Alnus rubra	+	+	+	+
Schoenoplectus acutus	+	-	-	-
Typha latifolia	o	-	-	-
Carex spp.	o	o	-	-
Rubus spectabilis	o	-	o	-
Rubus ursinus	o	+	o	o
Juncus spp.	-	o	-	-
Grasses (e.g., *Phalaris arundinacea*)	o	-	o	-

Note: predominant **+** ≥ 25% to100%; secondary 5% < **o** < 25%; and minor **-** < 5%

The percentage of vegetation cover (mean ± 95% CI) at egg-mass sites was 16 ± 4% (range 1 – 60%, n = 51). Mean vegetation cover trended similar at sites used by *R. aurora* (20 ± 5%, range 4 – 45%, n = 22) and *P. regilla* (22 ± 17%, range 1 – 60%, n = 8), and lower at *A. gracile* egg-mass sites (7 ± 3%, range 1 – 25%, n = 21) (Appendix, **Figure A**).

The stem count in the water column (i.e., stem density) at egg-mass sites (mean ± 95% CI) was 14 ± 4 (range 4 – 76, n = 60), after removal of one outlier from the plant-stem data during analysis (i.e., a stem-count estimate of 400 at a *P. regilla* egg-mass site that was more than four times higher than the nearest stem count). The mean stem count at *R. aurora* egg-mass sites was 15 ± 5 (range 5 – 69, n = 22) (**Figure 4**). In comparison, the mean stem count trended higher at sites with *P. regilla* egg masses (29 ± 21, range 8 – 76, n = 7) and lower at sites with *A. gracile* egg masses (11 ± 1, range 4 – 18, n = 31).

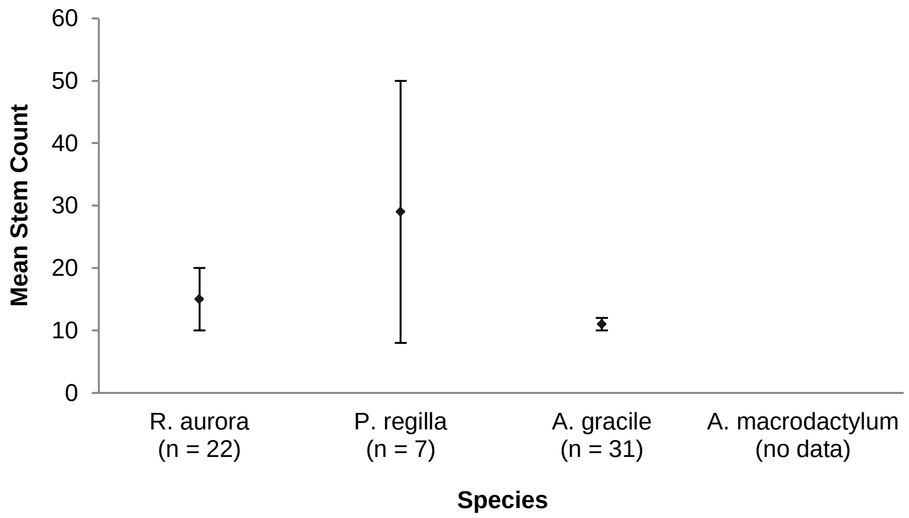

Figure 4. Mean (± 95% CI) stem count, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

The mean stem diameter (± 95% CI) of the vegetation occurring at egg-mass sites was 5.6 ± 0.4 mm (range 0.1 – 60.5 mm, n = 937). Stem diameters at egg-mass sites varied among species. For sites used by *R. aurora*, the mean stem diameter was 6.9 ± 0.7 mm (range 0.8 – 60.5 mm, n = 261), for *A. gracile* 4.6 ± 0.6 mm (range 0.4 – 56.5 mm, n = 291), and for *P. regilla* 2.6 ± 0.4 mm (range 0.2 – 12.2 mm, n = 110) (Appendix, **Figure B**). Similarly, the mean stem diameter of attachment substrates that I measured at remnant egg masses was smaller for *P. regilla* (1.4 ± 0.2 mm, range 1.1 – 1.5 mm, n = 4) compared with *A. gracile* (3.1 ± 0.6 mm, range 0.5 – 8.8 mm, n = 29).

The mean canopy closure (± 95% CI) at egg-mass sites was 69 ± 5% (range 0 – 100%, n = 192). Among species, the mean canopy closure trended higher at *A. gracile* egg-mass sites (92 ± 2%, range 37 – 100%, n = 84) and lower (with similar percentage values) at *P. regilla* (52 ± 14%, range 0 – 100%, n = 32) and *R. aurora* (55 ± 8%, range 5 – 69%, n = 88) egg-mass sites (Appendix, **Figure C**). Trends were similar when considering cardinal directions separately.

Solar radiation (i.e., photosynthetic photon flux density [PPFD]) trended opposite when compared with canopy closure. The mean solar radiation (± 95% CI) at egg-mass sites was 631 ± 89 µM/m^{2}s (range 11 – 2106 µM/m^{2}s, n = 192). Mean solar radiation was lower at *A. gracile* egg-mass sites (235 ± 99 µM/m^{2}s, range 11 – 1798 µM/m^{2}s, n = 84), compared with *P. regilla* (1136 ± 265 µM/ m^2s, range 29 – 2054 µM/m^2s, n = 32) and *R. aurora* (845 ± 166 µM/m^2s, range 31 – 2106 µM/m^2s, n = 88) egg-mass sites (Appendix, **Figure D**). The trend was similar when considering cardinal directions separately.

The results for the in-situ measurements of temperature, water chemistry, and water-level fluctuations for 2017 are in the Appendix, **Table A** and **Table B**. The water temperature (mean and SD) during oviposition (March and April) was 8.9 ± 3.7°C (range 4 – 17.1°C, n = 33), and in summer (June and July), 17.5 ± 4.2°C (range 16 – 24.2°C, n = 10). The four ponds held water throughout this study.

3.3 Vegetation structure and abiotic variables

The logistic regression model with backward elimination predicted the likelihood of an egg mass being present based on the independent variables (**Table 4**).

Table 4. Results from the logistic regression model with backward elimination for the likelihood that vegetation structure and abiotic variables (i.e., independent variables) affect egg-mass presence (i.e., dependent variable). Water depth (Depth.Bot) and stem count (Num.Stems) contribute significantly to the model (in bold). The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

Independent Variable	β Estimate	SE	z-value	p-value
Intercept	-9.617	2.330×10^{3}	-0.004	0.9967
Veg.Cover	-0.0299	0.0493	-0.607	0.5440
Veg.TypeAHerbEmer	18.77	2.330×10^{3}	0.008	0.9936
Veg.TypeAHerbRipa	36.50	6.926×10^{3}	0.005	0.9958
Veg.TypeAWoody	21.14	2.330×10^{3}	0.009	0.9928
Depth.Bot	**-8.460**	**2.223**	**-3.806**	**0.0001**
Num.Stems	**0.3160**	**0.1194**	**2.646**	**0.0081**
Stem.Dia	-0.0455	0.1097	-0.415	0.6785
Canopy.Close	-0.0923	0.0712	-1.295	0.1954
PPFD	0.0926×10^{-3}	1.384×10^{-3}	0.067	0.9467

Note: Under β estimate are the regression coefficients associated with the independent variables. The intercept represents the likelihood of egg-mass presence (EM.Presence) when the independent variables are zero (0). The quotient of β estimate and standard error (SE) produces the z-value. Total observations: 94. Null deviance: 130.31 (df = 93). Residual deviance: 44.58 (df = 84). Akaike Information Criterion: 64.58

Based on the model, stem count (Num.Stems) and water depth (Depth.Bot) significantly influenced the likelihood of egg-mass presence (**Figure 5** and **Figure 6**).

The logistic regression analysis shows the results of fitting the model:

$$\mathrm{EM.Presence} = \beta0 + \beta1 * (\mathrm{Num.Stems}) + \beta2 * (\mathrm{Depth.Bot}) + \varepsilon$$

Equation 2

$$\mathrm{EM.Presence} = -9.617 + 0.316 * (\mathrm{Num.Stems}) - 8.46 * (\mathrm{Depth.Bot}) + \varepsilon$$

Equation 3

Testing the performance of the model as a predictor revealed a model accuracy of 95.8% (**Table 5**, **Equation 4**). For the data set (n = 24), the model predicted “1” (i.e., egg mass present) 11 times, and “0” (i.e., egg mass not detected) 13 times. In actuality, an egg mass was present 12 times, and not detected 12 times.

Table 5. Confusion matrix describing the model accuracy on the testing data set (n = 24) based on the predicted classes (1 “egg mass present” and 0 “egg mass not detected”). The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

	Predicted		
Actual	Egg mass present (1)	Egg mass not detected (0)	Total Actual (n)
Egg mass present (1)	TP = 11	FN = 1	12
Egg mass not detected (0)	FP = 0	TN = 12	12
Total predicted (n)	11	13	

Note: TP = true positives (predicted 1, actual 1); TN = true negatives (predicted 0, actual 0); FP = false positives (predicted 1, actual 0); and FN = false negatives (predicted 0, actual 1).

$$\mathrm{Model\ Accuracy} = \frac{(\mathrm{FP} + \mathrm{TN})}{\mathrm{Total}} = \frac{(12 + 11)}{24} = 0.958 * 100\% = 95.8\%$$

Equation 4

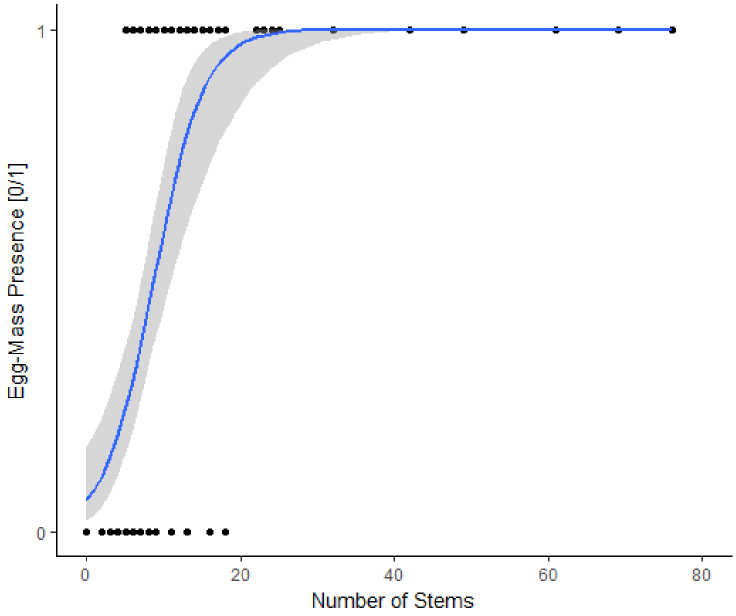

Figure 5. Curve of logistic regression model predicting the likelihood of an egg mass being present with number of stems (i.e., stem density) as independent variable (p = 0.008). The curve's positive slope shows that it is more likely for egg masses to be present at higher stem densities. Grey shading represents the standard error. Study location: Sechelt, B.C. Date: 2017.

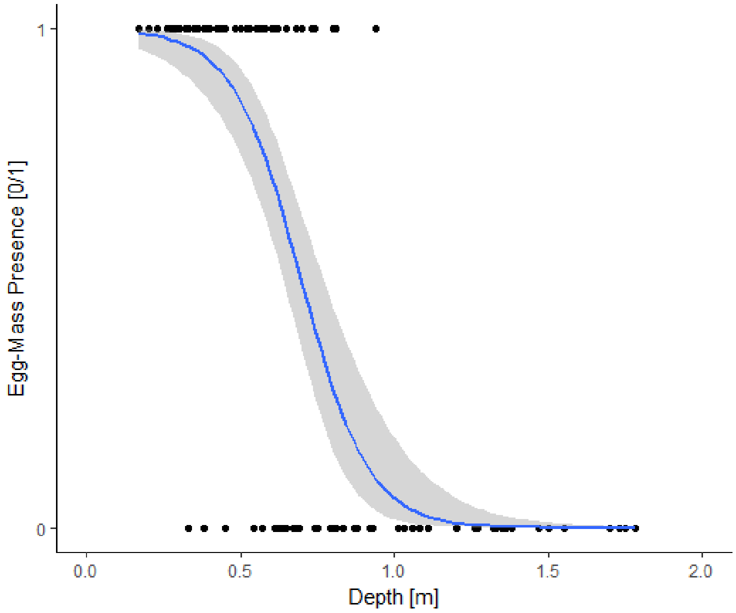

Figure 6. Curve of logistic regression model predicting the likelihood of an egg mass being present with water depth as independent variable (p = 0.0001). The curve's negative slope shows that it is more likely for egg masses to be present at shallower depths. Grey shading represents the standard error. Study location: Sechelt, B.C. Date: 2017.

4.0 Discussion

4.1 Vegetation structure: stem density

This study reveals that increasing the vegetation structure in ponds by increasing plant-stem density increases the likelihood of egg masses being deposited by amphibians (**Figure 5**). Pond-breeding amphibians have previously been shown to deposit more egg masses in ponds containing complex woody or herbaceous vegetation structure (Egan and Paton 2004). Proximity to suitable rearing areas for offspring also has been associated with oviposition-site selection (Refsnider and Janzen 2010). Amphibian species tend to differ in their life-history strategies, and the characteristics of oviposition sites used by any particular species potentially represent compromises based on different selective pressures (Wells 2007).

Rana aurora begin to deposit eggs at a water temperature of about 7°C, and once initiated, oviposition concludes within two weeks (Storm 1960, Licht 1969). In 2017, the first *R. aurora* egg masses at my study site were detected on 5 March (M. Blockberger, pers. comm.) and most hatchlings had emerged by the end of April. Hatchlings of *R. aurora* immediately gather below the egg mass, resting for one to two days in submerged vegetation or debris (Storm 1960). *Rana aurora* larvae feed on algae and use dense vegetation as refuge (British Columbia Ministry of Environment 2015). Their development rate is slow in the first two weeks, and *R. aurora* larvae are vulnerable to predation, e.g., by diving beetles (Storm 1960) or invasive bullfrogs (*Lithobates catesbeianus*) (Kiesecker et al. 2001). *Pseudacris regilla* oviposit in temperatures of 9 – 12°C (Brattstrom and Warren 1955). In 2017, egg masses first appeared between 20 March and 31 March at my site. Larvae of *P. regilla* feed on algae and develop and metamorphose rapidly in water temperatures above 12°C (Brattstrom and Warren 1955, Schaub and Larsen 1978, Weitzel and Panik 1993). Earlier metamorphosis could be advantageous because it leads to a longer post-transformation growth period before hibernation, resulting in larger individuals that are less vulnerable to starvation and desiccation (Schaub and Larsen 1978). Larvae that metamorphose early, however, might do so at a smaller size that can compromise fitness (Purrenhage and Boone 2009). *Pseudacris regilla* larvae are susceptible to predation, e.g., by garter snakes (i.e., *Thamnophis elegans* and *T. sirtalis*) (Schaub and Larsen 1978) and larvae of ambystomatid salamanders (Petranka 1998). *Ambystoma macrodactylum* breed between February and March (in Oregon, U.S.A.), and females oviposit within a few days after mating (Petranka 1998). In 2017, egg masses of this species were present at my site on 19 March. Following emergence after 2 – 5 weeks, *A. macrodactylum* hatchlings live in shallow water, hiding in sediment, under rotting leaves, logs or rocks (Corkran and Thoms 1996). From cover, hatchlings capture prey such as small invertebrates, while larvae consume a variety of aquatic organisms including invertebrates and *P. regilla* tadpoles (Petranka 1998). Their main predators are garter snakes and bullfrogs (Petranka 1998). *Ambystoma gracile* start depositing egg masses in March (B.C.) within a time span of 1 – 7 weeks (Petranka 1998). Egg masses of *A. gracile* were present at my site on 19 March 2017. Hatchlings emerge about six weeks after the first eggs have been deposited, and begin feeding on zooplankton soon after (Petranka 1998). Larvae of *A. gracile* mature after 1 – 2 years of growth in permanent or semi-permanent ponds, and increased food levels can reduce their time to metamorphosis (but not size at metamorphosis) (Petranka 1998). Larvae prey on larger amphipods, insect larvae, and tadpoles, and predators of hatchlings and larvae include beetle larvae and fish (Petranka 1998). In experiments on the influence of predators on anuran oviposition behaviour, 75% of individuals selected deposition sites without predators, while 9% selected sites with predators (Buxton and Sperry 2017). Potential predators of anurans and urodeles at my site include garter snake, great blue heron (*Ardea herodias*), and raccoon (*Procyon lotor*).

Amphibians might select oviposition sites in areas of higher stem density because these areas provide increased food resources and refuge for their offspring. Vegetation structure (in the form of live and artificial T*ypha* spp.) has previously been shown to have a positive effect on larval survival and species evenness in amphibian communities consisting of *Rana pipiens*, *Bufo americanus*, *Hyla versicolor*, and *Ambystoma maculatum* (Purrenhage and Boone 2009). This effect was observed even in the absence of predators, signifying that the presence of vegetation structure alone might induce increased foraging behaviour as a result of perceived safety (Purrenhage and Boone 2009). In addition to providing refuge from predators or competitors, periphyton growing on the vegetation (particularly live *Typha*) can increase the food availability for larvae (Purrenhage and Boone 2009). Live *Typha* plants provided more structural complexity, compared to artificial plants, because live plants grew during the experiment (Purrenhage and Boone 2009). The plant growth might have increased the stem density and surface area of the plants resulting in additional periphyton growth. In accordance with the general life-history strategies of the amphibians in this study, anuran larvae might use areas of higher stem density for food and refuge, whereas urodeles might use these areas mainly for refuge.

Percentage cover of vegetation has been identified as having a positive influence on egg-mass abundance (Egan and Paton 2004), while emergent vegetation cover has been positively associated with species richness (Burne and Griffin 2005, Shulse et al. 2012). Burne and Griffin (2005) attributed this positive association to a higher vegetative complexity. Mole salamanders (i.e., *Ambystoma* spp.) can show a negative association with the amount of aquatic vegetation in wetlands (Denton and Richter 2013). In contrast, Porej and Hetherington (2005) identified no association between species richness and the amount of emergent vegetation cover. In many of these studies, however, the term “vegetation cover” was not clearly defined, and this might explain the variable results. In my study, percent vegetation cover did not affect egg-mass deposition by amphibians, suggesting that vegetation density in the water column provides a greater advantage to amphibians depositing eggs than vegetation cover. One possible explanation is that vegetation cover represents the proportion of the pond-surface area concealed by a vertical projection of the vegetation (Robinson and Bolen 1984, Jennings et al. 1999), and not the vegetation structural complexity in the water column, and hence, vegetation cover might contribute less to the availability of structural complexity as refuge or food for larvae than stem density.

Vegetation structure also provides shade and attachment sites for egg masses (Egan and Paton 2004, Calhoun et al. 2014). Many amphibians (including the four species in this study) use the stems of vegetation for egg attachment. The vegetation types used for egg attachment in this study correspond with those of other studies. For example, egg-attachment substrates of *Rana sylvatica* and *A. maculatum* also include submerged live and dead woody shrubs, riparian vegetation and emergent vegetation (Egan and Paton 2004). Attachment substrates for *P. regilla* egg masses often are submerged grass blades, twigs or rushes (e.g., *Eleocharis palustris*) (Schaub and Larsen 1978, Corkran and Thoms 1996). *Ambystoma gracile*, *A. macrodactylum* and *R. aurora* have been reported to select breeding sites in thin-stemmed emergent vegetation consisting of *Juncus* spp., *Carex* spp., *Typha* spp., herbaceous plants and grasses (Licht

1969, Richter and Azous 1995). *Ambystoma macrodactylum* often attach their eggs singly or in loose, linear clusters to fine stems, twigs, or detritus (Corkran and Thoms 1996, Petranka 1998), while *A. gracile* use tree limbs, cattails and other vegetative support (Petranka 1998). When vascular plants are absent, coarse and small woody debris falling into the water body from adjacent trees and shrubs can serve as egg-attachment substrates and refuge from predators (Calhoun et al. 2014). While a few studies detected a strong relationship of *R. aurora* and *A. gracile* to micro-site-level vegetative characteristics in wetlands (Richter and Azous 1995, Pearl et al. 2005), others reported no evidence that pond-breeding amphibians selected certain vegetation for breeding (Lehtinen et al. 1999). In this study, vegetation type and stem diameter had no effect on the oviposition-site selection by amphibians. It appears that stems of various vegetation types with small stem diameters are suitable for egg attachment. Similarly, canopy closure and solar radiation did not affect egg-mass deposition. While I did not analyze this statistically, the trends in my data (i.e., lower canopy closure and higher solar radiation at egg-mass sites of *R. aurora* and *P. regilla*, and higher canopy closure and lower solar radiation at egg-mass sites of *A. gracile*) suggest that additional research could warrant investigating the differential use of canopy closure for oviposition between anurans and urodeles. *R. sylvatica* egg masses, for instance, have been documented to occur more predictively in ponds with greater canopy closure (Egan and Paton 2004). Canopy closure explained the differences in community composition between natural (i.e., higher canopy closure) and constructed (i.e., lower canopy closure) wetlands (Denton and Richter 2013). Changes in the stratification of the vegetation around ponds also can influence the species richness and assemblage of amphibians, indicating the benefit of vegetation heterogeneity including areas of variable canopy (Halverson et al. 2003, Lesbarrères et al. 2010, Klaus and Noss 2016).

My results demonstrate that quantifying plant stems (as a proxy of stem density) is useful for examining the vegetation structural complexity in the water column.

4.2 Abiotic variables: water depth

There is strong support from previous research that pond depth influences the presence, richness and diversity of amphibian species (Porej and Hetherington 2005, Lesbarrères et al. 2010, Denton and Richter 2013, Calhoun et al. 2014, Hossack 2017) and the selection of breeding sites by amphibians (Storm 1960, Licht 1969, Petranka

1998, Egan and Paton 2004, Rudolf and Roedel 2005, Tupper and Cook 2008, Indermaur et al. 2010, Hossack 2017). My study shows that shallower water depth increases the likelihood of egg masses being deposited (**Figure 6**). Changes in water depth have also been shown to influence oviposition behaviour of some amphibians. An increase in pond depth (i.e., by flooding) was associated with changes in colonization by amphibians and resulted in an increase of *Rana luteiventris,* no change in *A. macrodactylum* and *P. regilla*, and a decrease of *Anaxyrus boreas* (Hossack 2017).

The trends in my data indicate that the amphibians at my site use shallow pond areas ($\dot{x}$ = 0.44 m, 95% CI = 0.03 m) for oviposition (**Figure 3**). Previous studies have also suggested that *R. aurora* often deposit egg masses in quiet areas with depths of 30 cm or greater that receive ample amounts of sunlight (Storm 1960, Licht 1969). Less often, egg-mass sites of *R. aurora* are in deeper water (up to 1 m) and in partial shade (Licht 1969, British Columbia Ministry of Environment 2015). *Rana aurora* place their egg masses 15 cm – 40 cm below the water's surface but the egg masses tend to surface as water levels recede during development (Storm 1960). (Egan and Paton 2004) documented that *R. sylvatica* and *A. maculatum* selected shallow sections of ponds with water depths of less than 0.5 m for oviposition, and a similar water depth was used by *P. regilla* (Corkran and Thoms 1996) and *A. macrodactylum* (Corkran and Thoms 1996, Petranka 1998). *Ambystoma macrodactylum* often place their eggs within 5 cm – 8 cm of the water surface (Petranka 1998). *Ambystoma gracile* have been reported to deposit egg masses 0.5 m – 1 m (Petranka 1998) and sometimes up to 2 m (Corkran and Thoms 1996) below the surface, suggesting that suitable water depths at egg-mass sites could exceed 1 m.

One possible explanation for the selection of shallow water for oviposition is that shallower areas have warmer temperatures that optimize egg development (e.g., growth rate and timing of emergence). Temperature, particularly for ectotherms such as amphibians, drives physiological processes and can influence survival (Freidenburg and Skelly 2004). Warmer temperatures have been shown to accelerate developmental rates for embryos and tadpoles of *P. regilla* (Schaub and Larsen 1978) and other amphibian species (Halverson et al. 2003, Indermaur et al. 2010). In contrast, sudden freezing temperatures experienced by early ovipositing species (e.g., *A. macrodactylum* and *R. aurora*) can lead to failure of the eggs or death of the offspring (Corkran and Thoms 1996). Canopy-mediated light can also influence water temperature. In an experiment with *R. sylvatica,* larvae in closed-canopy ponds selected areas where pond temperatures were warmer than those expected by chance, while larvae in open-canopy ponds were distributed at random (Freidenburg and Skelly 2004). Researchers in Europe revealed that the two factors most influencing the breeding-site selection in *Bufo b. spinosus*, *B. viridis*, *Rana temporaria*, and *R. latastei* were water depth and predation risk (Indermaur et al. 2010). Although breeding-site selection differed among species, their results indicated that structurally complex ponds, in combination with warm temperatures, had the highest species diversity.

Hence, the selection of shallower water for oviposition could also be related to the amount of vegetation structure (i.e., stem density) of the plant species that are able to colonize these shallow areas, together with warmer temperatures, amount of refuge, and food resources. Dissolved oxygen in the water column might also be a consideration because both amphibian eggs and larvae require oxygen for development, by diffusion through the cell or body wall (eggs and larvae) or via the use of gills or lungs (larvae) (Wells 2007). Egg masses deposited in shallow water, however, can likely maintain sufficient dissolved oxygen levels throughout development because oviposition typically occurs early in spring when water temperatures are relatively cold and oxygen-saturated (Wells 2007).

My results agree with other studies (Rudolf and Roedel 2005, Indermaur et al. 2010, Groezinger et al. 2012, Peterman et al. 2014), in suggesting that the effects of the biotic and abiotic environment jointly influence oviposition-site selection by amphibians.

4.3 Management considerations

Data collection should be repeated at least once to determine between-year variation in the data and increase the robustness of the data set. This would also enable the use of the data from the second year to test the logistic regression model. For this one-year study, I was able to apply a portion of my data set to test the model accuracy because my sample size was sufficiently large.

Marking egg-mass locations with flagging during egg-mass counts would be desirable if among-year comparison of egg-mass abundance was a management objective. Flagging of egg masses is time intensive but avoids double counting between survey rounds (Egan and Paton 2004), possibly leading to better accuracy. Flagging would need to occur in a way that does not compromise the structural complexity of the vegetation. Because the timeframe for my study was one year, I used the highest count from three distinct survey rounds for analysis. Although I possibly missed egg masses that were deposited by amphibians early or late in the breeding period, using the highest yield prevented double counting.

Conducting egg-mass counts or vegetation sampling by boat would be advantageous in larger ponds or wetlands to enable access to areas that are too far from shore or too deep to access on foot. I considered using a boat for access to deeper sections (>1.2 m) but wind, debris and boat instability prevented me from maneuvering, or holding the boat in position, and, therefore, I abandoned this method. Although it is possible that I missed egg masses as a result, I am confident that I covered about 90% of the ponds.

Although it is possible to conduct egg-mass surveys and vegetation sampling concurrently, the disturbance of the egg masses might lead to egg-mass detachment from stems and potential failure and should be avoided. I measured biotic (i.e., vegetation) variables in June. This timing avoided disturbing egg masses because most amphibian larvae had already emerged. This timing also meant that the emergent vegetation (e.g., *Typha* and *Schoenoplectus*) had started to grow and expand. I was able to distinguish new growth (green) from older plants (brown) based on colour. To avoid overestimating the amount of vegetation, I documented live and dormant plants separately and only used measurements for dormant plants in my analyses.

If future data collection is to include photosynthetically active radiation, data for each site should be collected with the quantum meter in a set (longer) time frame when sun intensity is similar (e.g., between 11:00 and 15:00 hrs). Setting a longer time frame with similar sun intensity considers fluctuations in measurements based on short-term changes in sun exposure, potentially reducing among-site variation and enabling analysis of cardinal directions separately. To increase precision, I pooled the data from the four cardinal directions and used the mean value for the logistic regression analysis.

Sampling at the 1x1-m plot level is an ecologically relevant spatial resolution for understanding the effect of the biotic and abiotic variables on oviposition-site selection by amphibians. For the statistical analysis, I chose the 1-m^2 plot level and pooled the data from the 25-cm^2 subplots where I observed multiple egg masses. I achieved this by taking means of the data and only considering one egg mass at each 1 m^2 plot.

4.4 Implications for restoration

The results of this study show that stem density and water depth influence oviposition-site selection by amphibians. Both of these independent variables are useful metrics that enable a restoration manager to examine the suitability of the vegetation structural complexity and amount of shallow areas in ponds for pond-breeding amphibians. If the restoration goal is to increase the number of potential egg-deposition sites for pond-breeding amphibians, a manager can focus on quantifying stems and measuring water depth at the 1x1-m plot level. Entering the data (for stem count and depth) into the logistic regression model (**Equation 3**) will enable a manager to calculate the likelihood ratio of egg-mass presence. The model is a valuable tool that predicts egg-mass presence, based on these two variables, with over 95% accuracy.

Vegetation structure needs to be a central component in the restoration and management of breeding sites for amphibians (Purrenhage and Boone 2009), along with water depth (Egan and Paton 2004). Higher stem density (i.e., mean stem count ≥ 10 in the water column) and shallower water (i.e., mean depth ≤ 0.5 m) increased the likelihood of egg-masses being present at my study site. Based on these results, I suggest that managers consider stem density and water depth when planning to restore breeding ponds for native amphibians. Consideration of these variables can potentially lead to increased amphibian abundance and diversity and contribute to the success of restoration projects.

5.0 Conclusions

Stem density and water depth significantly affect oviposition-site selection by amphibians. My research hypothesis that amphibians select oviposition sites in areas of increased vegetation structural complexity was supported in that a higher stem density increased the likelihood of egg masses being present. Quantifying stems in the water column might characterize vegetation structure better than estimating percent cover of vegetation. My hypothesis that amphibians select shallow pond areas (i.e., ≤ 0.5 m) for oviposition over deeper areas was supported in that shallower water ($\dot{x} = 0.44$ m, 95% CI = 0.03 m) increased the likelihood of egg-mass presence. Vegetation structure has previously been shown to affect the selection of egg-deposition sites by amphibians (Egan and Paton 2004) but this study also indicates that stem counts, as a proxy for stem density, can be used to quantify vegetation density in the water column. Stem counts are useful for characterizing the vegetation structural complexity in ponds at the 1x1-m plot level.

Vegetation structural complexity might mediate species’ interactions (e.g., predation) and community composition (e.g., species richness and evenness) by providing refuge and food resources (Purrenhage and Boone 2009). Oviposition in structurally complex vegetation might, therefore, create an advantage for the offspring. Warmer temperatures in shallow water areas possibly accelerate egg-development rates, as has been shown for embryos and tadpoles (Schaub and Larsen 1978, Halverson et al. 2003, Indermaur et al. 2010). Hence, restoration projects that incorporate shallow and more structurally complex areas into their pond designs can increase the abundance and diversity of amphibian communities, contributing to the success of restoration projects.

While pond-level investigations are central to examining the requirements of pond-breeding amphibians for oviposition, other amphibian stages should also be considered in restoration objectives, including larval requirements for successful metamorphosis (Purrenhage and Boone 2009, Shulse et al. 2012) and the broader riparian forest surrounding ponds for dispersing metamorphs (Egan and Paton 2004, Brown et al. 2012). Trends in my data suggest that further research is warranted to examine the differential use of canopy closure for oviposition of anurans and urodeles.

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6.1 Personal communication

Blockberger, M. 2017. Manager, Sunshine Coast Botanical Garden, Sechelt, B.C.

Appendix

Supplemental Data

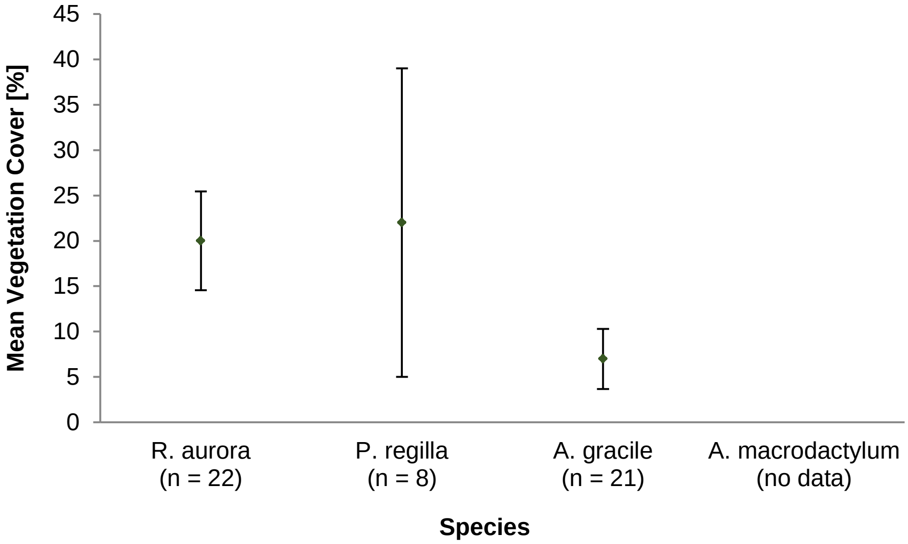

Figure A. Mean (± 95% CI) vegetation cover, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

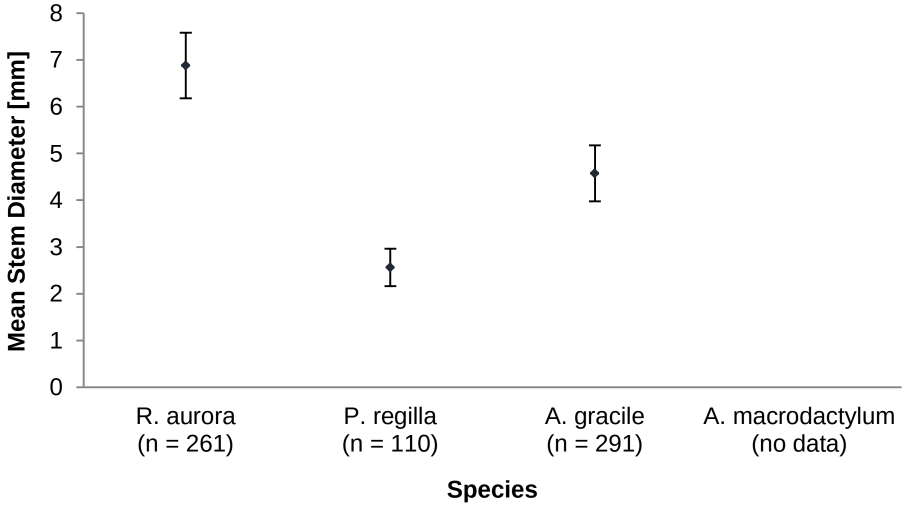

Figure B. Mean (± 95% CI) stem diameter, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

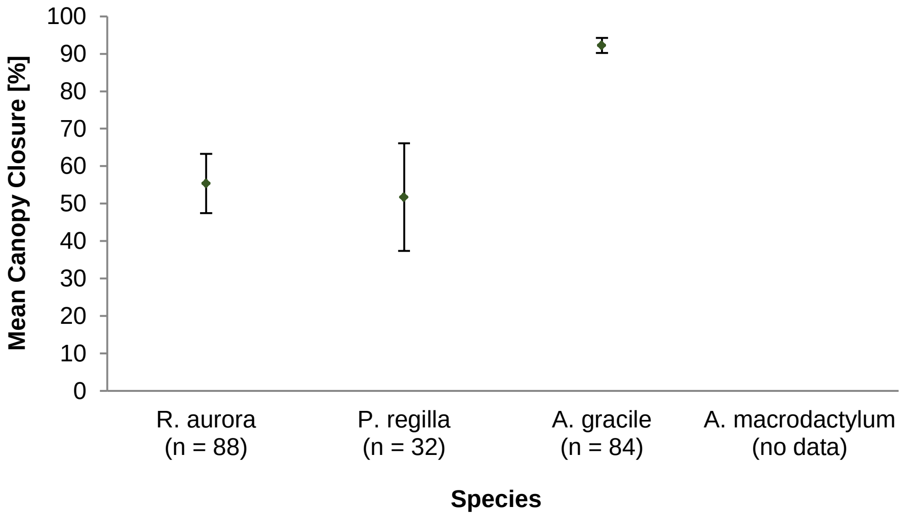

Figure C. Mean (± 95% CI) canopy closure, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

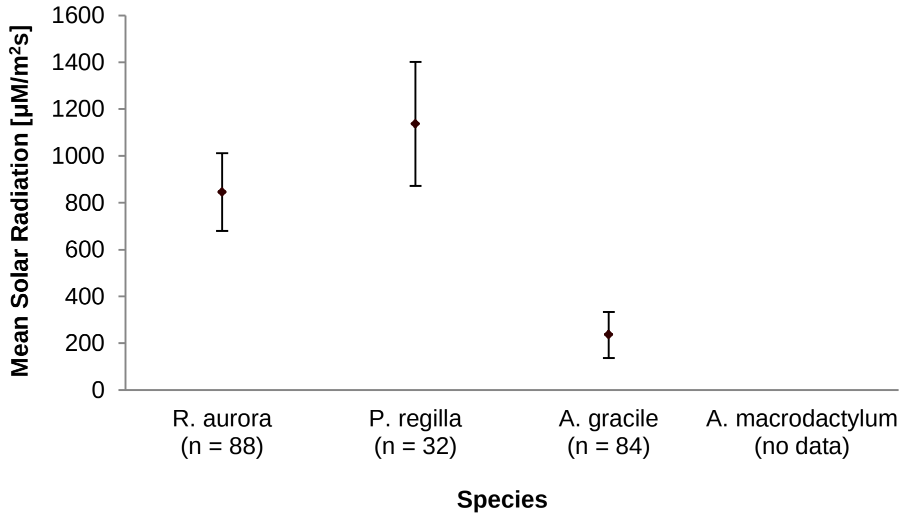

Figure D. Mean (± 95% CI) solar radiation, by species, recorded at egg-mass sites. The study was conducted in 2017 at the Sunshine Coast Botanical Garden, Sechelt, B.C.

Table A. Summary of mean temperature and water chemistry (in-situ) for each study pond (2017), Sunshine Coast Botanical Garden, Sechelt, B.C. Temperature includes water and air. Water chemistry includes pH, electric conductivity (EC) and dissolved oxygen (DO). The standard deviation (SD) for the samples (n>1) is provided in parenthesis.

Pond	Month	n	Temp. Water (°C)	Temp. Air (°C)	pH	EC (µS/cm)	DO (mg/L)
Dragonfly	March	4	7.9 (±2.1)	10.5 (±2.9)	6.3 (±0.2)	129.0 (±2.9)	
	April	3	16.5 (±0.7)	17.0 (±0.0)	6.4 (±0.1)	114.0 (±0.0)	10.8
	June	2	19.6 (±4.6)	22.0 (±5.7)	6.8 (±0.2)	126.5 (±0.7)	
	July	1	24.2	26.0	7.4	174.0	
Frog	March	4	7.4 (±1.0)	10.0 (±2.3)	6.3 (±0.4)	80.0 (±7.4)	
	April	3	13.6 (±0.4)	16.0 (±0.0)	6.6 (±0.1)	81.0 (±1.2)	9.0
	June	1	15.7	19.0	6.9	103.0	
	July	1	23.0	29.0	6.6	105.0	
North	March	5	7.6 (±1.3)	11.8 (±1.0)	6.5 (±0.3)	65.0 (±3.1)	
	April	2	12.6 (±1.3)	16.0 (±1.4)	6.8 (±0.1)	62.0 (±2.1)	5.3
	June	1	15.9	23.0	6.5	70.0	
	July	1	17.3	22.0	6.8	71.0	
Towhee	March	9	5.4 (±0.9)	9.8 (±1.5)	6.0 (±0.3)	86.0 (±36.3)	
	April	3	9.8 (±0.9)	14.0 (±1.4)	6.6 (±0.4)	78.0 (±26.3)	5.9
	June	2	13.0 (±1.0)	17.5 (±2.1)	6.9 (±0.1)	87.0 (±27.6)	
	July	1	13.7	21.0	6.4	109.0	

Table B. Water-level fluctuations from May to December 2017, Sunshine Coast Botanical Garden, Sechelt, B.C. The maximum pond level in May (i.e., full pool) was used as the reference against which all measurements were compared.

	Change in Water Levels (m)						
Pond	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Dec**
Dragonfly	0	-0.161	-0.328	-0.243	-0.448	-0.382	-0.007
Frog	0	-0.045	-0.184	-0.348	-0.493	-0.367	-0.019
North	0	-0.162	-0.313	-0.491	-0.637	-0.575	-0.328
Towhee	0	-0.086	-0.284	-0.481	-0.649	-0.586	-0.186

Note: Dragonfly Pond frequently received water (from North Pond) in summer to prevent it from drying. All four ponds contained water throughout 2017. No measurements were taken in November.